An unusual pattern of ligand-receptor interactions for the α7 nicotinic acetylcholine receptor, with implications for the binding of varenicline

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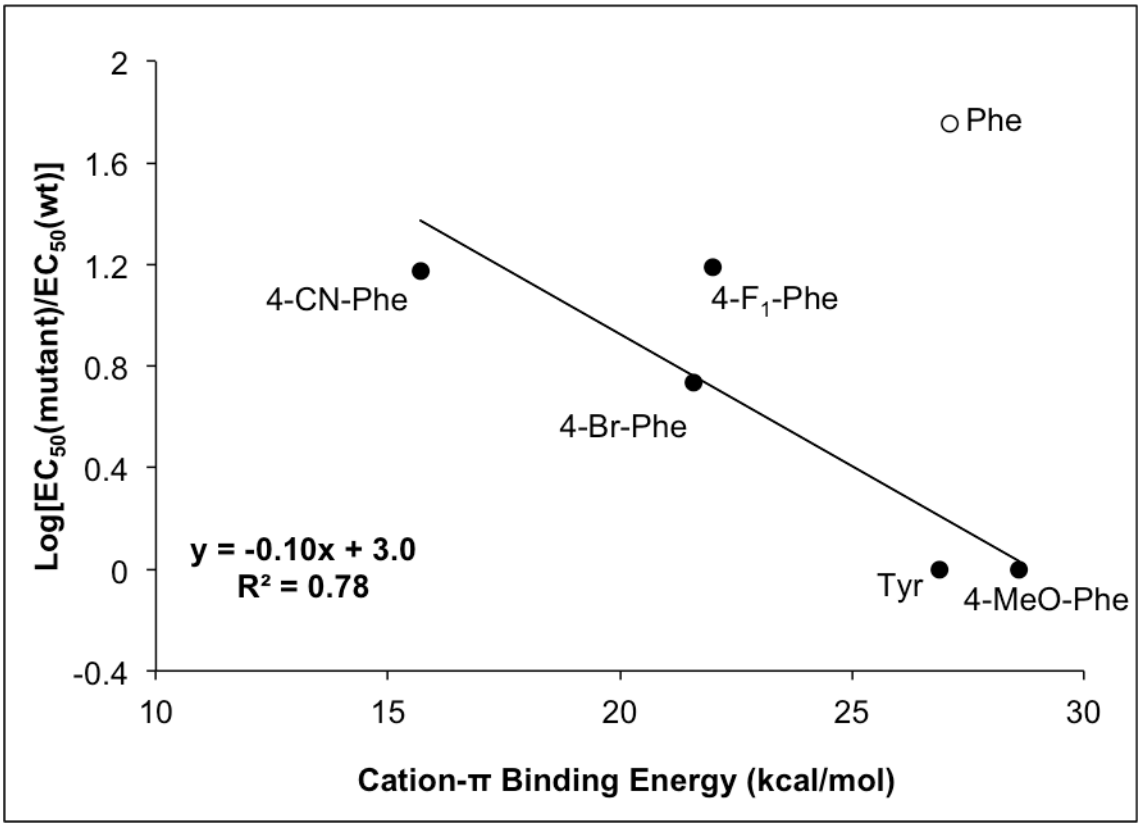
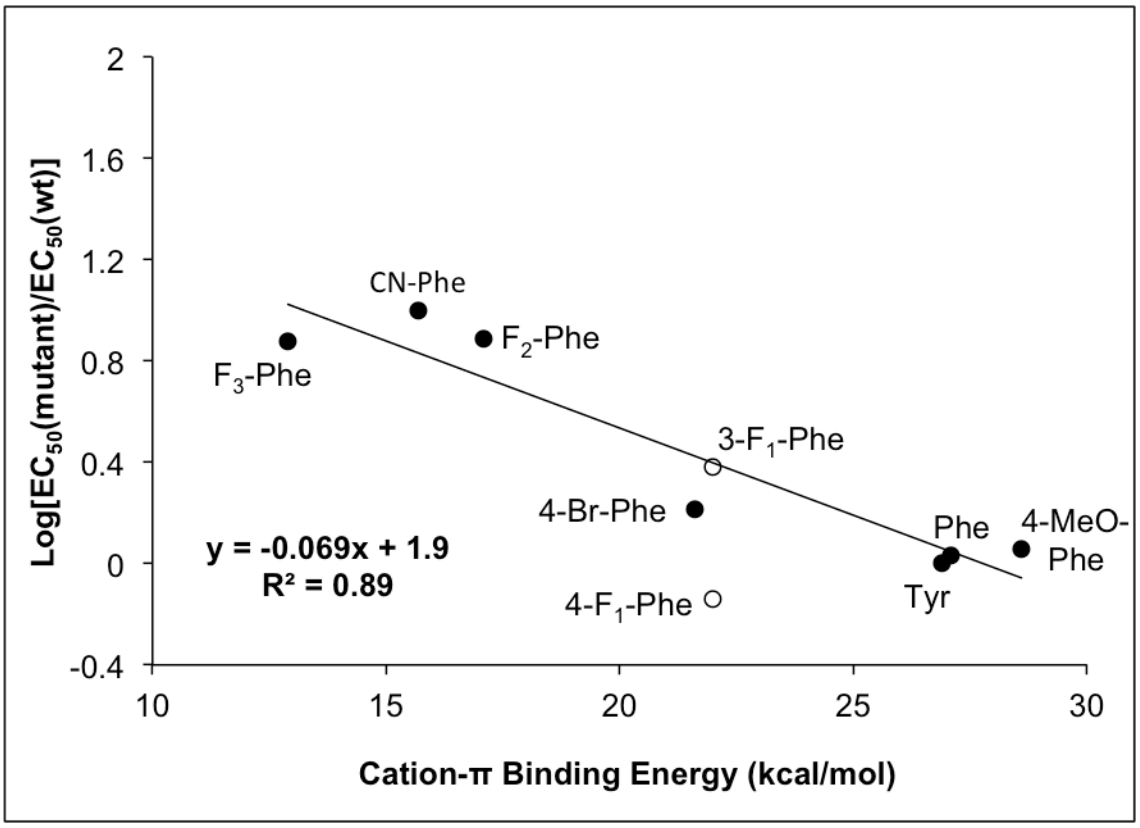
Supplemental Fig. 1. Cation-π binding plot for TyrA, in which log[EC_{50}(mutant)/EC_{50}(wt)] is plotted *versus* quantitative cation-π binding energies (Lummis et al., 2005; Zhong et al., 1998); a strong linear correlation would suggest a cation-π interaction. Phe (open circle) is not included in the fit.



Supplemental Fig. 2. Cation-π binding plot for TyrC2, in which log[EC_{50}(mutant)/EC_{50}(wt)] is plotted *versus* quantitative cation-π binding energies (Lummis et al., 2005; Zhong et al., 1998); a strong linear correlation would suggest a cation-π interaction. 3-F_1-Phe and 4-F_1-Phe (open circles) are not included in the fit.



References:

Lummis SC, D LB, Harrison NJ, Lester HA and Dougherty DA (2005) A cation-pi binding interaction with a tyrosine in the binding site of the GABAC receptor. *Chem Biol* **12**:993-997.

Zhong W, Gallivan JP, Zhang Y, Li L, Lester HA and Dougherty DA (1998) From ab initio quantum mechanics to molecular neurobiology: a cation-pi binding site in the nicotinic receptor. *Proc Natl Acad Sci U S A* **95**:12088-12093.

Supplemental Table 1. Wild type and principal face mutations. EC_{50} and Hill coefficient (n_H) are ± SEM for goodness of fit to the Hill equation.

Mutation	Agonist	EC_{50} (μM)	Fold Shift	n_H	n
wt	ACh	99 ± 3		2.7 ± 0.2	16
wt	Varenicline	1.99 ± 0.03		2.8 ± 0.1	15
wt	Epibatidine	0.34 ± 0.01		3.0 ± 0.2	13
Y93 Tyr	Varenicline	2.21 ± 0.05		3.0 ± 0.2	7
Y93 Phe	Varenicline	126 ± 5	57	2.4 ± 0.2	9
Y93 4-F_1-Phe	Varenicline	34 ± 3	15	2.0 ± 0.3	11
Y93 F_2-Phe	Varenicline	>100			8
Y93 F_3-Phe	Varenicline	>100			8
Y93 4-Br-Phe	Varenicline	12 ± 1	5.4	3.1 ± 0.6	7
Y93 4-CN-Phe	Varenicline	33 ± 2	15	2.5 ± 0.4	10
Y93 4-MeO-Phe	Varenicline	2.19 ± 0.05	0.99	2.3 ± 0.1	6
W149 Trp	Varenicline	2.5 ± 0.1		2.1 ± 0.1	17
W149 F_3-Trp	Varenicline	4.1 ± 0.1	1.6	2.3 ± 0.1	15
W149 F_4-Trp	Varenicline	9.6 ± 0.8	3.8	2.0 ± 0.2	15
W149 5-CN-Trp	Varenicline	2.1 ± 0.1	0.84	2.6 ± 0.4	12
Y188 Tyr	Varenicline	2.23 ± 0.08		2.4 ± 0.2	8
Y188 Phe	Varenicline	>100			6
Y195 Tyr	Varenicline	2.12 ± 0.02		2.76 ± 0.06	8
Y195 Phe	Varenicline	2.28 ± 0.09	1.1	3.1 ± 0.4	6
Y195 3-F_1-Phe	Varenicline	5.1 ± 0.07	2.4	1.9 ± 0.02	12
Y195 4-F_1-Phe	Varenicline	1.53 ± 0.05	0.72	3.4 ± 0.3	9
Y195 F_2-Phe	Varenicline	16.3 ± 0.7	7.7	2.4 ± 0.2	12
Y195 F_3-Phe	Varenicline	16 ± 1	7.5	2.6 ± 0.3	7
Y195 4-Br-Phe	Varenicline	3.48 ± 0.07	1.6	3.6 ± 0.2	5
Y195 4-CN-Phe	Varenicline	21 ± 1	9.9	3.8 ± 0.9	11
Y195 4-MeO-Phe	Varenicline	2.42 ± 0.09	1.1	2.8 ± 0.3	11
S150 Thr	Varenicline	0.81 ± 0.04		2.2 ± 0.2	11
S150 Tah	Varenicline	2.4 ± 0.1	3.0 from Thr	2.2 ± 0.2	15
K145A	ACh	N.R.			
K145Q	ACh	590 ± 20	6.0	2.5 ± 0.1	8
K145Q	Varenicline	9.4 ± 0.2	4.7	1.6 ± 0.2	7
K145R	ACh	1600 ± 100	16	2.6 ± 0.3	6
K145R	Varenicline	61.6 ± 0.2	31	3.19 ± 0.03	9

Supplemental Table 2. Complementary face mutations. EC_{50} and Hill coefficient (n_H) are ± SEM for goodness of fit to the Hill equation.

Mutation	Agonist	EC_{50} (μM)	Fold Shift	n_H	n
V108 Val	ACh	103 ± 2		2.9 ± 0.2	10
V108 Vah	ACh	184 ± 9	1.8	2.3 ± 0.2	10
V108 Val	Varenicline	2.3 ± 0.1		4 ± 1	8
V108 Vah	Varenicline	10.0 ± 0.5	4.3	2.5 ± 0.3	9
V108 Val	Epibatidine	0.396 ± 0.005		3.02 ± 0.09	7
V108 Vah	Epibatidine	0.64 ± 0.04	1.6	3.4 ± 0.5	11
L119 Leu	ACh	120 ± 6		2.5 ± 0.3	9
L119 Lah	ACh	180 ± 8	1.5	2.4 ± 0.2	6
L119 Leu	Varenicline	2.26 ± 0.02		2.76 ± 0.05	7
L119 Lah	Varenicline	3.15 ± 0.08	1.4	2.2 ± 0.1	9
L119 Leu	Epibatidine	0.290 ± 0.005		3.3 ± 0.1	8
L119 Lah	Epibatidine	0.75 ± 0.01	2.6	3.4 ± 0.1	10
N107L	ACh	350 ± 3	3.5	2.48 ± 0.05	5
N107L	Varenicline	2.77 ± 0.05	1.4	2.6 ± 0.1	4
N107L	Epibatidine	1.37 ± 0.02	4.0	2.44 ± 0.06	4
W55A	ACh	134 ± 8	1.4	1.9 ± 0.2	8
W55A	Varenicline	67.6 ± 0.9	34	2.95 ± 0.09	11
W55A	Epibatidine	5.8 ± 0.3	17	2.2 ± 0.2	10
L109A	ACh	303 ± 7	3.1	2.4 ± 0.1	9
L109A	Varenicline	0.53 ± 0.01	0.27	3.3 ± 0.2	12
L109A	Epibatidine	0.282 ± 0.008	0.83	2.8 ± 0.2	9
Q117A	ACh	180 ± 4	1.8	2.7 ± 0.1	10
Q117A	Varenicline	4.85 ± 0.06	2.4	2.88 ± 0.09	12
Q117A	Epibatidine	0.90 ± 0.05	2.6	3.0 ± 0.4	11
L119A	ACh	210 ± 9	2.1	2.3 ± 0.2	11
L119A	Varenicline	41 ± 1	21	2.6 ± 0.2	11
L119A	Epibatidine	3.20 ± 0.09	9	3.0 ± 0.2	10